EVALUATION OF SERUM AND SALIVARY ALKALINE PHOSPHATASE LEVELS IN PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY: A COMPARATIVE STUDY

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CERTIFICATE

This is to certify that **Dr. M.J.RENGANATH,** Post Graduate student (2014-2017) in the Department of Periodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319, has done this dissertation titled **"EVALUATION OF SERUM AND SALIVARY ALKALINE PHOSPHATASE LEVELS IN PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY: A COMPARATIVE STUDY"** Under our direct guidance and supervision in partial fulfilment of the regulations laid down by the Tamilnadu Dr.M.G.R Medical University, Chennai – 600032 for MDS., (Branch-II) Periodontology degree examination.

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DECLARATION

TITLE OF THE DISSERTATION	Evaluation of serum and salivary alkaline phosphatase levels in patients with chronic periodontitis before and after non-surgical periodontal therapy: A comparative study
PLACE OF THE STUDY	Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319
DURATION OF THE COURSE	3 years
NAME OF THE GUIDE	Dr.T. Ramakrishnan, MDS.,
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I hereby declare that no part of the dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319. In addition, I declare that no part of this work will be published either in print or in electronic media without the guides who has been actively involved in dissertation. The author has the right to reserve for publish work solely with the permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319

Co-Guide **Guide & Head of department**

Signature of candidate

ABSTRACT

BACKGROUND:

Diagnosis of the active phase of periodontal disease and identifying the patients at risk for active disease is being a challenge for clinical investigators. The traditional method of diagnosing periodontitis one includes assessment of clinical parameters and radiographic aids to evaluate the periodontal tissue destruction. Saliva has the potential to be used as the diagnostic fluid for oral disease. Its easy method of collection through non-invasive methods. Evaluation of ALP by simplified method of spectrometry and its cheaper analysis cost makes the biomarker ALP as a resilient and reliable one for the diagnosis of periodontal disease activity

AIM:

The aim of this study is to compare the quantitative levels of Alkaline phosphatase in saliva and serum before and after scaling and root planing in patients with chronic generalised periodontitis.

MATERIALS AND METHODS:

A total number of 50 subjects (40 with chronic generalised periodontitis and 10 periodontally healthy volunteers) of 30 to 50 years were included in the study. After getting the informed consent signed, all the individuals participated in the study were subjected to measurement of clinical parameters such as OHI-S, Gingival index, probing depth and CAL and then saliva and blood sample collection was done and analysed for ALP levels by spectrometry. The clinical parameters, saliva and serum ALP were re-evaluated after 30 days following phase 1 periodontal therapy. The results were statistically analysed using paired t test and One-way ANOVA.

RESULTS:

The saliva and serum ALP levels were significantly increased in patients with chronic generalised periodontitis with increase in clinical

parameters such as OHI-S, Gingival index, probing depth and CAL when compared with periodontally healthy individuals. The saliva and serum ALP levels were significantly decreased following phase 1 periodontal therapy along with improvement in clinical parameters thus exhibiting a positive correlation between clinical parameters with both saliva and serum ALP levels. P values from the statistical tests presented were found to be statistically highly significant at P-value .000**

CONCLUSION:

With the limitations of the present study it could be concluded that ALP levels in saliva can be used for the diagnosis of active phase of periodontal disease and also for evaluation of the treatment outcomes following periodontal phase 1 therapy.

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LIST OF ABBREVATIONS

ACP	:	acid phosphatase
ALP	:	alkaline phosphatase
ALT	:	alanine aminotransferase
AST	:	aspartate transferase
BOP	:	bleeding on probing
BUN	:	blood urea nitrogen
Ca	:	calcium
CAL	:	clinical attachment level
CEJ	:	cementoenamel junction
CI-S	:	calculus index score
CK	:	creatine kinase
CPITN	:	community periodontal index of treatment needs
CS	:	chondroitin sulphate
DI-S	:	debris index score
DNA	:	deoxyribose nucleic acid
ED	:	estrogen-deficient
ES	:	estrogen-sufficient
GCF	:	gingival crevicular fluid
GGT	:	gamma glutamil transferase
GI	:	Gingival Index
HH	:	hypergonadotropic hypogonadism
IFCC	:	International Federation of Clinical Chemistry
IL-1β	:	Interleukin-1β
K	:	Potassium
LDH	:	lactate dehydrogenase
OHI-S	:	oral hygiene index-simplified
SRP	:	scaling and root planing
UNC-15	:	University of North Carolina-15

INTRODUCTION

Chronic periodontitis has been defined as “an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss.

Although periodontitis is an infectious disease of gingival tissue origin, changes that occur in the bone are crucial as the alveolar bone destruction is responsible for tooth loss. The most common cause of alveolar bone destruction in periodontitis is the extension of inflammation from the marginal gingiva to the underlying periodontal tissues.[1]

Biochemical markers can detect inflammatory changes in short period of time whereas longer period is required to detect measurable changes in bone density using radiographs.

Salivary constituents for diagnosing periodontal disease includes enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products, ions, and volatile compounds.[2]

Intracellular enzymes are released progressively into the gingival crevicular fluid (GCF) and saliva from the damaged cells of periodontal tissues. Several enzymes that are evaluated for the early diagnosis of periodontal disease includeslactate dehydrogenase (LDH), aspartate and alanine aminotransferase (AST, ALT), creatine kinase (CK), alkaline and acid phosphatase (ALP, ACP), and gamma glutamiltransferase (GGT).

ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules and is a marker of bone metabolism. It is a membrane-bound glycoprotein produced by various number of cells, such as polymorphonuclear leukocytes, macrophages, fibroblasts and osteoblasts, within the area of the periodontium and gingival crevice.[3]

Studies demonstrated that the elevated serum ALP level is associated with patients with chronic kidney disease.[4] Studies have also shown increased ALP activity in Post-menopausal women with periodontitis compared with non-periodontitis individuals.[5]

ALP is very important enzyme in the periodontium, as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis.

Various studies have assessed the levels of salivary ALP with respect to gingivitis, chronic periodontitis and correlation with clinical parameters. But there is lagging evidence regarding the comparative effects of ALP in serum and saliva following periodontal treatment.

Totan et al. [6] investigated the influence of periodontal disease on ALP, aspartate aminotransferase (AST), aminopeptidase, and glucuronidase. Salivary samples were evaluated in patients with periodontal disease showed that periodontal destruction along with the probing depth, gingival bleeding index, and suppuration were related to elevated ALP levels in saliva. These results were also supported by the

findings in the study conducted by **Todorovic et al.** [7] that there was an increased activity of salivary ALP in patients with periodontal disease in relation to periodontally healthy group. The study group further showed a positive correlation between the salivary enzyme activity and gingival index values thereby indicating that ALP levels of an individual may best serve as a marker in periodontal treatment planing and monitoring.[8,9]

ALP levels in saliva can be detected same as serum ALP estimation by using UV spectrometry and its analysis is cost-effective. So far, ALP has not been supported by research findings as a predictive indicator for future periodontal tissue breakdown. The purpose of this study is to compare the Serum and Salivary ALP levels in chronic periodontitis patients before and after periodontal phase 1 therapy which serves to hold to the hypothesis that saliva can be used as an alternative to serum for evaluating ALP as a biomarker in periodontal disease progression.

AIM AND OBJECTIVES

The aim of this study is to compare the quantitative levels of ALP in serum and saliva before and after scaling and root planing in patients with chronic generalised periodontitis.

For this purpose, the following objectives were undertaken:

1. To evaluate and compare the saliva and serum ALP levels in chronic generalized periodontitis patients with that of periodontally healthy individuals.

2. To compare and correlate the clinical parameters such as OHI-S, gingival index, probing depth and CAL with biomarker levels of serum and salivary ALP in patients with chronic generalized periodontitis at baseline and following phase 1 periodontal therapy.

GENERAL REVIEW

ALP is a membrane-bound glycoprotein produced by numerous cells, such as osteoblasts, macrophages, polymorphonuclear leukocytes and fibroblasts within the area of the periodontium and gingival crevice. ALP activity in serum has been extensively studied for the past few years, and it was suggested that ALP allows bone mineralization by releasing an organic phosphate, contributing to the deposition of calcium-phosphate complexes into the osteoid matrix. ALP might also promote mineralization by inorganic pyrophosphate hydrolyzation, which acts as a potent inhibitor of hydroxyapatite crystal mineralization and dissolution, within the extracellular calcifying matrix vesicles.[10]

In general, accelerated bone loss is related to an increased bone turnover rate, accompanied by increased levels of serum and urine biochemical markers of bone turnover, such as collagen cross-links, alkaline phosphatase (ALP), and osteocalcin.

ALP activity is mostly present in all the organs of the body and is especially associated with membranes and cell surfaces located in mucosa of small intestine and proximal convoluted tubules of the kidney, in bone (osteoblasts), liver and placenta. Although the metabolic function of the enzyme is not understood, it appears that ALP is associated with lipid transport in the intestine and with the calcification process in bone.[11]

DIAGNOSTIC SIGNIFICANCE OF ALP

Higher levels of ALP is having more value for diagnostic significance in the evaluation of hepatobiliary and bone disorders. In hepatobiliary disorders, elevations are seen more predominantly in obstructive conditions than in hepatocellular disorders. In bone disorders, elevations are seen when there is involvement of the osteoblasts.

In biliary tract obstruction, ALP levels range from 3 to 10 times due to increased synthesis of the enzyme induced by cholestasis. In contrast, 3 fold increase is seen in hepatocellular disorders like hepatitis and cirrhosis.

The bone isoenzyme increases due to potent osteoblastic activity and also the levels are normally elevated in children during periods of growth and in adults older than age 50 years, where an elevated ALP level may be difficult to interpret.

Individuals who have blood group B or O shows presence of intestinal ALP isoenzyme in serum. Furthermore, in these individuals, increases in intestinal ALP occur after consumption of a fatty meal. Increased levels are also found in patients undergoing chronic hemodialysis.

In normal pregnancy, increased ALP activity, averaging one and one-half times can be detected between 16-20 weeks of pregnancy. ALP activity increases and persists until the onset of Labor and then

returns to normal within 3 days to 6 days. Elevations also may be seen in complications of pregnancy such as hypertension, preeclampsia and eclampsia, as well as in threatened abortion.

Elevated ALP levels may be seen in a variety of bone disorders. Conceivably the highest elevations of ALP activity occurs primarily in Paget's disease (osteitisdeformans). Other bone disorders including rickets,osteomalacia, hyperparathyroidism, and osteogenic sarcoma were also documented with increased ALP levels. In addition, increased levels are seen in the case of healing bone fractures and during periods of physiologic bone growth. [13]

ALP levels are significantly decreased in the inherited condition of hypophosphatasia. Subnormal activity is due to the absence of the bone isoenzyme and results in inadequate bone calcification.

ALP levels have often been evaluated in gingival crevicular fluid (GCF) to validate the relationship between periodontal conditions and disease activity.[12,13]

Plagnat et al.[14] studied ALP in GCF from implants with and without peri-implantitis and suggested that ALP could be a promising marker of bone loss around dental implants. **Gibert et al.** [15] studied the activity of ALP in serum from patients with chronic periodontitis and showed a relationship between loss of attachment in periodontal disease and ALP activity in serum. **Binder et al.** [13] performed a longitudinal study of eight patients and demonstrated a positive correlation between GCF ALP concentration and attachment loss.

REVIEW OF LITERATURE

The diagnosis of active phases of periodontal disease and the identification of patients at the risk for active disease are challenges for clinical investigators and practitioners alike. Researchers are confronted with the need for innovative diagnostic tests that focus on the early recognition of the microbial challenge to the host. Optimal innovative approaches would correctly determine the presence of current disease activity, for future periodontal breakdown and to evaluate the response to periodontal interventions. Hence, a new paradigm for periodontal diagnosis would ultimately improve the clinical management of patients with periodontal disease. [16]

SALIVA IN THE FIELD OF DIAGNOSIS: [17]

Analysis of blood and its compounds has been used for several decades for lab diagnostic procedures. However, other biological fluids are also utilized frequently for the diagnosis of disease, for example, urine, cerebrospinal fluid and thus saliva which could offer some distinct advantages in certain situations.

Electrolytes enter the saliva via osmotic gradients and are regulated by rate of secretion, nature of stimulus and level of mineralocorticoids in the circulation. Because serum components of saliva are derived primarily from local vasculature that originates from the carotid arteries, saliva has a prodigious fluid source that provides many, if not most, of the same molecules found in the systemic

circulation. This makes saliva as a potentially valuable fluid for the diagnosis of various systemic diseases.

There are 3 major salivary glands (Parotid, submandibular and sublingual) that secrete saliva into the oral cavity. Saliva from these glands provides different mixtures of serous and mucinous derived fluid and is primarily useful for the detection of gland specific pathology.

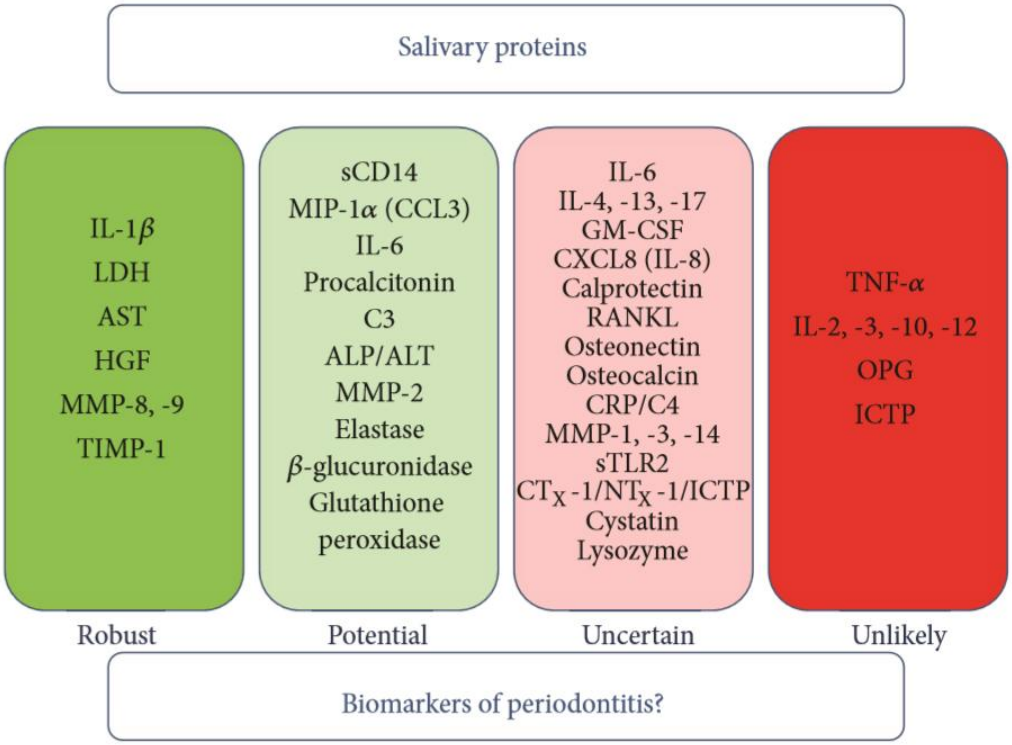
Whole saliva, by contrast is composed by a mixture of oral fluids from the major salivary and minor salivary glands and also contains constituents of non salivary origin, including Gingival Crevicular Fluid (GCF), serum transudate from the mucosa and sites of inflammation, epithelial and immune cells, food debris and many microbes. Whole saliva is most frequently studied because, its collection is easy, non-invasive and rapid to obtain without the need for specialised equipment.

Unstimulated saliva is commonly collected by the draining method, where the subject's head is tilted forward so that saliva moves towards the anterior region of the mouth and the pooled saliva is drooled into a wide sterile container. To date, unstimulated whole saliva has been used in the majority of diagnosis studies.

SALIVARY BIOMARKERS OF PERIODONTAL DISEASE:

Saliva is appealing for use as a diagnostic fluid for point of care analysis for oral related diseases, because it is rapid, easy and non-

invasive to collect and generally readily abundant. However, few studies have longitudinally monitored salivary biomarker profiles in patients with respect to periodontal status or determined if salivary biomarkers accurately represent periodontal disease status over time, its concentration of analytes should diminish in response to periodontal therapy.[18]



"**Robust biomarkers**" are defined as those salivary proteins which have been shown to discriminate between periodontitis and oral health in at least 3 cross-sectional studies and for which there may be supporting evidence from longitudinal studies investigating the natural course of periodontitis and for the effects of treatment on biomarker levels.

"**Potential biomarkers**" are defined using identical criteria to robust biomarkers with the exception that there are 2 replicated cross-sectional studies showing disease discrimination in addition to possible supporting evidence from longitudinal studies but for which there may be limited contradictory studies. It is accepted that the entries in the robust and potential categories may be interchangeable depending on the existence of further studies which remain unpublished for commercial reasons.

"**Uncertain biomarkers**" are proteins for which there are only single study showing discrimination of periodontitis or for which there are several studies from which the evidence is contradictory.

"**Unlikely biomarkers**" are those proteins for which there are 3 or more studies which fail to provide evidence for an association with periodontitis in the absence of any evidence to the contrary. [19]

MARKERS OF ALVEOLAR BONE LOSS:

Many different biomarkers associated with bone formation, resorption and turn over such as Alkaline Phosphatase, Osteocalcin, Osteonectin and collagen telopeptidases have been evaluated in the GCF and saliva. These mediators are associated with local bone metabolism (in periodontitis) as well as with systemic conditions (Osteoporosis or Metastatic bone cancers). [16]

Badersten A et al., 1981 [20]

Investigated healing after non-surgical periodontal therapy in patients with periodontal pockets depth of 4-7mm in 15 patients. Patients were treated by supra and subgingival scaling either with hand or ultrasonic instruments. Clinical parameters like plaque scores, bleeding on probing, probing pocket depths and probing attachment levels were evaluated. There was improvement in clinical parameters during the initial 4–5 months after start of therapy and then a little change occurred during the rest of the 13-month observation period. Total of 106 sites demonstrated probing pocket depths ≥ 6 mm. At 13 months only 13 such sites were observed. The results revealed that the conservative treatment of patients with 4–7 mm deep pockets shown better clinical outcomes in the study, provided with the raise of question to what extent nonsurgical therapy is feasible also in patients with severely advanced lesions.

Isidor F et al., 1984 [21]

Evaluated the effect of root planing as compared to that of surgical periodontal treatment in 17 patients with advanced periodontal disease. Following initial examination, the teeth were scaled and the patients were given instruction in performing proper oral hygiene. The hygienic phase for the individual patient was continued until less than 20% of the tooth surfaces demonstrated plaque at 2 succeeding appointments. After re-assessment of the periodontal status, 1 side in both the maxilla and mandible was treated with modified Widman flap surgery. In I of the remaining quadrants, in the maxilla or mandible,

reverse bevel flap surgery was used. Bone contouring was not performed in any of the surgical procedures. The last quadrant was subjected to meticulous root planing under local anaesthesia. Subsequently, the patients were recalled every second week for professional tooth cleaning. The periodontal status of each patient was assessed at 3rdand 6th months following treatment. Clinical gain of attachment was obtained following all 3 modalities, but root planing resulted in slightly more gain of attachment than the 2 surgical procedures.

Ramfjord SP et al., 1987 [22]

Assessed in a clinical trial over 5 years results following 4 different modalities of periodontal therapy (Pocket elimination or reduction surgery, modified Widman flap surgery, Subgingival curettage & Scaling and root planing) in 90 on a random basis to each of the 4 quadrants of the dentition. Following professional tooth cleaning and oral hygiene instructions every 3 months, the pocket depth and attachment levels were scored once a year. 72 patients who completed the 5yrs observation were shown that for 1-3mm probing depth, scaling and root planing as well as subgingival curettage led to significantly less attachment loss than pocket elimination and modified Widman flap surgery. For 4-6mm pockets, scaling and root planing and curettage had better attachment results than pocket elimination surgery for 7-12mm pockets, thus concluded that clinical significance of SRP &subgingival curettage in treatment of deep periodontal pockets.

Greenstein G 1992 [23]

Addressed the advantages and limitations of non-surgical periodontal therapies to treat patients with mild to moderate chronic periodontitis by controlled clinical trials and assessed the efficacy of following treatment methods like mechanical instrumentation, ultrasonic debridement, supragingival and subgingival irrigation along with local drug delivery and systemic antibiotic therapy, host modulation therapy and concluded that most patients with mild to moderate periodontitis can be treated with non-surgical therapy.

Haffajee AD et al., 1997 [24]

Examined the effect of SRP on clinical and microbiological parameters in 57 subjects with adult periodontitis. Clinical and microbiological examination was done prior to and 3, 6 and 9 months after full-mouth SRP. Clinical parameters included plaque, redness, suppuration, BOP, pocket depth and attachment level. Subgingival plaque samples for the presence and levels of 40 subgingival taxa were determined using whole genomic DNA probes and checkerboard DNA-DNA hybridization. Sites with pre-therapy pocket depths of <4 mm showed a non-significant increase in pocket depth and attachment level. 4–6 mm pockets showed a significant decrease in pocket depth and a non-significant gain in attachment post-therapy, while 6 mm pockets showed a significant decrease in pocket depth and attachment level measurements post-therapy. Clinical improvement post-SRP was accompanied by a modest change in the subgingival microbiota, primarily a reduction in *P. gingivalis, B. forsythus and T. denticola,*

suggesting potential targets for therapy and indicating that radical alterations in the subgingival microbiota may not be necessary or desirable in many patients.

Cobb CM 2002 [25]

Analysed the Egyptian hieroglyphics and medical Papyri to evaluate the clinical significance of non-surgical therapy: the collective evidence from numerous clinical trials reveals a consistency of clinical response in the treatment of chronic periodontitis by SRP remains the "gold standard" thus controlling sub-gingival bacterial populations, removal of calculus, root smoothness and also improvement in clinical parameters like probing depth, attachment levels, bleeding on probing and gingival inflammation. It was also added that SRP acts as a significant component on a relatively new paradigm of complete mouth disinfection in a compressed time frame.

Hue AC et al., 1961 [26]

Investigated the acid and alkaline phosphatase activity on both whole blood and blood serum in 100 normals and 100 cases of periodontal disease and oriented the clinical data with chemical data. The results shown that there was no significant difference between the acid phosphatase of the normal cases and periodontal cases in either whole blood or in plasma. The ALP levels however was higher in the blood in periodontal cases suggested the positive correlation of ALP with respect to periodontal disease.

Ishikawa I et al., 1970 [27]

Investigated the correlation between Alkaline Phosphatase (ALP) level of activity in Gingival exudate and various clinical parameters, collected from incisor and canine region in 21 patients aged 18-65 years with extensive gingival inflammation and periodontitis. Clinical parameters included PMA index, Gingival index, periodontal pocket. Radiographic evaluation for bone loss by Marshall-Day and Shourie (1949). ALP was measured in 10µl of fluid and 20µl of serum by colorimetric technique. The results of analysis showed positive correlation between ALP levels in GCF with that of alveolar bone loss in patients with periodontitis along with increased clinical parameters concluded stating the prime relevance of ALP levels for diagnosing periodontal disease.

Miglani DC et al., 1974 [28]

Investigated the relationship of ALP to periodontal disease by collecting saliva from 45 patients with gingivitis and periodontitis and assayed for phosphatase activity by King & Armstrong method. Periodontal status of the patients was assessed using Russel's periodontal index and gingival inflammation and periodontal index were correlated with ALP activity. Based on the results, it was evident that with gingivitis, the ALP activity increases. It was then concluded that there is a definite rise in ALP activity with an increase in gingival score.

Chapple IL et al., 1994 [29]

Investigated GCF ALP levels in the health and in the presence of gingivitis in 30 patients. In gingival health, there was a site specific pattern of ALP concentration with higher enzyme concentrations around the upper and lower anterior teeth. Furthermore, clinically normal sites that had been subjected to different levels of plaque control produced significantly different ALP levels, which indicates that the biochemical components of GCF may be used to measure the sub-clinical inflammatory status. The ratio of GCF to serum ALP varied from 6:1 to 11:1 suggesting that the major source of enzymes is through local production and analysis of plaque within the study group demonstrated very low levels of ALP, indicating that the enzyme is likely to be largely derived from the periodontal tissues.

Chapple IL C et al., 1999 [3]

The study aimed to apply a novel enhanced chemiluminescence assay in the analysis of GCF ALP levels from 3666 sites in 25 patients with untreated adult periodontitis. Parameters assessed includes relative attachment level, probing pocket depth, bleeding on probing, GCF volume (µl), total ALP levels (µIU/30 s sample time) and ALP concentration (IU/l). Results shown that there were significant increase in total ALP levels and GCF volumes for active sites between baseline and 3 months measures; but there was no increase in the ALP concentration for control sites or test sites. Thus indicating that the total GCF ALP levels may serve as a predictor of future or current disease activity rather than the ALP concentration levels.

Gibert P et al., 2003 [15]

The author studied the activity of ALP isoenzyme among the other isoforms in the serum of 83 patients (59 with periodontal disease-chronic periodontitis, 24 as control group) by determining the total serum ALP activity and percentage of the different isoforms (bone, kidney and intestinal type) by Ektachem analyser and Gel agarose electrophoresis respectively. The comparison between the two groups resulted a relationship between loss of attachment in periodontal disease and a drop in bone ALP activity in serum. Moreover, these results suggested a gender based difference as well, with lower activity more frequent in women than in men.

DaltabanÖ et al., 2006 [30]

The study aimed at determining how estrogen status may possibly influence GCF ALP levels in estrogen-deficient (ED) and estrogen-sufficient (ES) post-menopausal women at baseline and one year after periodontal phase-I treatment in 36 post-menopausal women who were divided into two groups; chronic periodontitis and clinically healthy patients. The levels of ALP in GCF were measured photometrically and other clinical parameters were also measured at baseline and 1year post operatively. Periodontitis group demonstrated significantly increased GCF volumes and GCF ALP levels compared to control group. One year after periodontal treatment, the GCF volume, total GCF ALP and concentrations decreased significantly in both periodontitis group (ES/P; ED/P). Thus suggesting that the presence of ALP in GCF is not simply a reflection of the local inflammation state

and that a patient's estrogen status may possibly influence local ALP levels in GCF.

Todorovic T et al., 2006 [7]

The authors examined the activity of CK, LDH, AST, ALT, GGT, ALP and ACP in saliva from 30 patients with periodontal disease before and after phase I periodontal treatment and from 20 healthy individuals. Periodontal disease was determined based on clinical parameters (Gingival Index, bleeding on probing and probing depth). The obtained results shown statistically significant increase of activity of CK, LDH, AST, ALT, GGT,ALP and ACP in saliva in patients with periodontal disease in relation to control group along with a positive correlation between activity of salivary enzymes and value of Gingival index and there was significant decrease in activity of all salivary enzymes after treatment. Hence it was assumed that the activity of these enzymes in saliva, as biochemical marker for periodontal tissue damage, may be useful in diagnosis, prognosis and evaluation of therapy effects in periodontal disease.

Desai S et al., 2008 [31]

The purpose of the study was to evaluate ALP levels in saliva of 120 patients with chronic periodontitis who were assigned as 40 in each of 3 groups C0, C3 and C4 based on their largest community periodontal index of treatment needs CPITN code. Unstimulated saliva was collected and analysed with auto analyser using IFCC method in order to quantify their ALP concentrations in saliva in each group. It

was concluded that the ALP levels in saliva was increased with the increase in the CPITN score. Group C0 had the least while group C4 had the highest ALP levels. Hence, concluded that the salivary ALP levels may be useful as a potential bone turn over marker to establish the diagnosis and prognosis of periodontal disease.

Perinetti G et al., 2008 [32]

The study aimed to improve the understanding of how the healing of chronic periodontitis following scaling and root planing (SRP) affects GCF ALP activity after 15 days and 60 days in 16 sytemically healthy patients with moderate to advanced generalised chronic periodontitis. 92 pockets were randomized at the split mouth level with half receiving SRP and other half left untreated. Plaque index, probing depth, CAL, BOP were recorded at baseline, after 15 days and 60 days. GCF was collected from each pocket included in the study at the 3 time points for ALP estimation. Results shown a large and significant decrease in GCF ALP activity in 15 days after SRP with improvement in clinical parameters after 60 days, an increase in GCF ALP back to baseline levels was recorded along with further improvements in clinical parameters. Hence, it was concluded that GCF ALP reflects the short term periodontal healing/ recurrent inflammation phases in chronic periodontitis patients.

Üsal B et al., 2008 [33]

The study aimed at determining the possible relationship between alkaline phospahatase levels in the GCF and periodontal disease in men

with hypergonadotropic hypogonadism (HH) in 41 patients divided into 4 groups. 9 with HH and periodontitis, 11 with HH and gingivitis, 9 with systemically healthy and periodontitis, 12 with systemically healthy and periodontally healthy individuals. Clinical evaluation included Plaque index, gingival index, probing depth and CAL. The ALP IN GCF were measured by ELISA. The concentrations and total amounts of ALP in GCF were significantly higher in both periodontitis group compared to healthy and gingivitis groups. The serum ALP levels were significantly higher in the HH and periodontitis group when compared to the other groups. Thus it was concluded that HH could be implicated as a contributing factor to the progress of periodontal disease with increased ALP levels.

Malhotra R et al., 2010 [34]

The study aimed to assess the total activity of ALP in the GCF collected from healthy sites, sites with gingivitis and with chronic adult periodontitis along with an attempt made to establish the correlation of ALP activity with Plaque index, gingival Index, bleeding index and probing depth in 18 patients, divided into 3 groups as healthy sites-Group I; Gingivitis- Group II and periodontitis- Group III. ALP levels in GCF of all the 3 groups was determined by spectrophotometric analysis. The results shown that the total enzymatic activity of ALP was significantly higher in periodontitis as compared with healthy and gingivitis sites and was positively correlated with probing depth. Thus it was concluded that ALP can be considered as

periodontal disease marker as it can distinguish between healthy and inflamed sites.

Perozini C et al., 2010 [35]

Evaluated the levels of Interleukin-1β (IL-1β) and ALP in GCF and correlate these measurements with clinical characteristics of 36 individuals subdivided as 3 group healthy individuals and patients with gingivitis and periodontitis. GCF samples were obtained from 2 sites for each patient and were measured using Periotron 8000 whereas IL-1β levels were evaluated using the ELISA and ALP was measured by the kinetic method. The amount of ALP differed significantly among the 3 groups. The amount of IL-1β in periodontitis group was significantly higher than in the other groups, but no significant difference was found between the control group and the gingivitis group. There was no evidence for correlation between IL-1β and ALP levels thus concluding that monitoring immune markers may give additional information on healthy or diseased sites.

Jaiswal G et al., 2011 [36]

The study was carried out to compare ALP levels in cirrhosis patients with and without periodontitis and to correlate ALP levels with the severity of periodontitis in 30 liver cirrhosis patients with or without periodontitis. The parameters recorded includes OHI-S index, gingival index and CAL along with standardized panaromic radiographs to assess alveolar bone height. The total serum ALP level was determined with the kinetic method. The alveolar bone loss, CAL and

mean serum ALP level in the test group was higher than the control group and the difference was statistically significant. The older age group liver cirrhosis patients exhibited higher values for bone loss, CAL and serum ALP levels. Hence, it was concluded that there is a strong positive correlation between periodontal breakdown and serum ALP level in liver cirrhosis patients.

Dabra S et al., 2012[37]

The purpose of the study was to determine the salivary levels of ALP and ACP activities in patients with periodontal disease, before and after periodontal treatment. The experimental group consisted of 20 gingivitis patients, 20 periodontitis patients and the control group had 20 healthy subjects. The stimulated saliva was collected and analysed using automatic analyser. Periodontal disease was determined based on clinical parameters such as gingival index, probing depth and CAL. Patients with periodontal disease were under conventional periodontal treatment. The obtained results showed statistically significant increased activity of ALP and ACP in saliva from patients with periodontal disease in relation to control group and also a significant reduction in the enzyme levels after conventional periodontal therapy, suggesting that salivary ALP and ACP can be considered to be the biomarkers for evaluating periodontal tissue damage.

Kunjappu JJ et al., 2012 [38]

The study compares the levels of GCF ALP in patients with chronic periodontitis before and after scaling and root planing in 20 patients with localised periodontitis. The GCF was collected from the affected site prior to scaling and root planing for ALP estimation. The probing depth and plaque index at the sites were also measured at baseline, 7, 30 and 60 days for re-assessment. The obtained results showed a sustained, statistically significant decrease after treatment and thee was a positive correlation with probing depth but not with plaque index, measured at each interval. It was thus concluded that the assessment of level of periodontal disease and effect of mechanical plaque control on progression and regression of the disease can be evaluated precisely by the corresponding GCF ALP levels. Hence, ALP level is not only a biomarker for pathology, but also an indicator of prognosis of periodontitis.

Trivedi D et al., 2012 [39]

The study aimed to estimate the LDH, AST, ALP and CK enzymes in unstimulated saliva for valuing their importance as routine screening tool in diagnosis of periodontitis in patient group population of 205 males and 72 females with mean age of 37.5±6.9 years and control group of 95 healthy age matched adult volunteers. Subjects having probing depth of >3.5 mm diagnosed as periodontitis. Salivary level of LDH, CK, AST, and ALP were measured using commercially available kits developed for routine blood test. The results signified increased activities of enzymes in unstimulated saliva, which is either

derived from degenerating gingival tissue or systemic circulation due to infectious change in membrane permeability, thus increased levels of intracellular enzymes LDH, AST, ALP in unstimulated saliva can be used for the diagnostic tool in screening of periodontal disease.

Sanikop S et al., 2012 [40]

The study aimed to determine the presence and levels of ALP activity in GCF in periodontal health, gingivitis and chronic periodontitis from 45 sites, which were equally divided into 3 groups. Various clinical parameters like Gingival index, plaque index, probing pocket depth, CAL were also evaluated and correlated with the GCF ALP levels. The results showed that the difference between the mean ALP levels between the healthy and gingivitis group was found to be non-significant and that between the chronic periodontitis group and healthy as well as gingivitis group was found to be highly significant along with significant correlation existing between ALP levels and gingival index, probing depth as well as CAL. The findings suggests that and confirms the relationship between ALP levels and periodontal disease, thus indicating that GCF ALP levels can be used as potential biochemical marker for detection and progression of periodontal disease.

Ramesh A et al., 2013 [5]

The study aimed at the estimation of salivary ALP levels in 40 subjects, 20 in each groups in the age group of 50- 60 years. Group I is of 20 post-menopausal women without chronic periodontitis and group

II of 20 post-menopausal women with chronic periodontitis. The results shown significant increase in alkaline phosphatase in post-menopausal women with chronic periodontitis. Hence it was concluded that ALP can be used as a diagnostic marker of periodontitis in post-menopausal women; however ALP cannot solely be responsible for periodontitis, but it can be used as an addition aid in diagnosing periodontitis.

Khongkhunthian S et al., 2014 [41]

The study compared two biochemical markers Chondroitin sulphate (CS) and alkaline phosphatase (ALP) levels in GCF samples collected from patients with various degrees of disease severity. The samples includes 10 patients with gingivitis (50 sites); 33 patients with chronic periodontitis (50 sites) as well as 10 healthy volunteers (50 sites) by periopaper strips and levels of CS and ALP measured by an ELISA and a fluorometric assay. The results demonstrated low levels of CS and ALP in non-destructive and slightly destructive periodontitis sites, whereas significantly high levels in moderately and severely destructive sites. Stronger correlates were found between CS levels and periodontal parameters including probing depth, loss of clinical attachment levels, gingival index and plaque index, than between ALP levels and these parameters suggested that CS level is a better diagnostic marker than ALP level for evaluating disease severity of chronic periodontitis.

Perumal CL et al., 2014 [42]

The study was carried out to compare the serum total ALP level among 31 healthy individuals and 36 chronic periodontitis patients in Tamilnadu and to evaluate the racial behaviour in the enzyme levels. Comparison of the total ALP activity between the healthy and chronic periodontitis group showed an increase in total ALP activity among chronic periodontitis. Thus, the results were similar to other studies, suggestive of using serum ALP measurement as a reliable assay for chronic periodontitis and there was no racial or ethnic differences.

Caúla AL et al., 2015 [43]

The study aimed to evaluate the relationship between severe chronic periodontitis and serum creatinine and ALP levels. 100 patients were evaluated, 66 with severe chronic periodontitis and 34 periodontally healthy individuals. Blood samples were collected after an overnight fast and serum creatinine and ALP levels were determined. The results showed that the patients with periodontitis exhibited a lower mean creatinine level and higher mean alkaline phosphatase levels than in control group. Also there was significant correlation between the periodontal parameters and serum creatinine and ALP levels. Hence it was concluded that severe chronic periodontitis was associated to lower creatinine and higher ALP levels.

Luke R et al., 2015 [44]

The study aimed to estimate the levels of enzymes AST, ALT, ALP and BUN in saliva and to correlate it with that of clinical

parameters to saliva of healthy subjects, gingivitis patients and patients with chronic periodontitis. The study included 40 male subjects of age group 21 to 50 years. Clinical parameters like OHI-S, sulcus bleeding index, probing depth, CAL and periodontal index were recorded. The results showed significant increase of activity of AST, ALT, ALP and BUN in saliva from patients with periodontal disease in relation to gingivitis and control group. There was also an increase in periodontal parameters with an increase in salivary enzymes. Thus it was concluded that salivary enzymes can be used as biomarker to determine periodontal tissue damage, which may be useful in diagnosis, prognosis and evaluation of post therapy effects in periodontal disease.

Soud P et al., 2015 [45]

The study aimed to determine the enzyme and mineral activity in serum of subjects with chronic periodontitis namely ALP, ACP, Ca and K in 30 patients. Blood samples were taken to assess the levels of enzymes and minerals. Periodontal disease was identified clinically by assessing probing depth and CAL. Amount of bone loss was confined to radiographic evaluation. The results showed that the periodontitis patients had high level of ALP which indicates statistically significant results as compared to ACP levels, which had no significance with the results. From this study, it was concluded that serum level of ALP was significantly elevated in patients with chronic periodontitis and can be considered as a biomarker for periodontal disease activity.

MATERIALS AND METHODS

A clinical study was conducted at the department of periodontology, Adhiparasakthi dental college and hospital (APDC&H), Melmaruvathur. A total number of 50 subjects in the age range of 30 to 50 years were selected from the out-patient division of Periodontics, APDC&H. Ethical clearance for the study was obtained from the Institutional review board, APDC&H (Reference No:2014-MD-BrII-SAS-05). All the subjects participated in the study were informed about the nature of the study and all the participants signed an informed consent form.

Method:

Sample size:

- Control Group:10 individuals (periodontally healthy individuals)
- Study Group:40 patients (chronic generalised periodontitis)

Inclusion criteria:

- Control group: Participants with at least 20 natural teeth and probing pocket depth of 2-3mm with no attachment loss and bleeding on probing with< 20% sites.
- Study group: Participants had to have 5 qualifying sites in 2 quadrants with a minimum of 2 affected teeth in each quadrant with each site having probing depth ≥5mm, CAL ≥3mm, bleeding on probing.

Exclusion criteria:

- Any systemic diseases
- Smokers
- Pregnancy
- Has not undergone any periodontal therapy for the past 1 year.
- Patients who are not maintaining their oral hygiene.

The following clinical indices and parameters were recorded:

- OHI-S Index(Greene & Vermillion)
- Gingival Index(Loe&Silness)
- Probing pocket depth
- CAL

The following biochemical markers were analysed:

- Salivary ALP level
- Serum ALP level

Method to be followed:

After getting the informed consent signed, all the individuals participated in the study were subjected to measurement of clinical indices including OHI-S and Gingival Index and clinical parameters including Probing depth and CAL at baseline for the study group, followed by saliva and blood sample collection for both study and control groups, which was then analysed for ALP estimation. Following sample collection, complete ultrasonic scaling to be performed at day 1 for patients under study group (Chronic periodontitis). Complete Root

planing should be completed within 15 days from the baseline in two subsequent visits. On 30th day after completion of phase 1 periodontal therapy, patients are reviewed where saliva and blood samples are collected and analysed again for ALP activity.

OHI-S (Greene and Vermillion, 1964) [46]

The Simplified Oral Hygiene Index (OHI-S) differs from the original OHI (The Oral Hygiene Index) in the number of the tooth surfaces scored (6 rather than 12), the method of selecting the surfaces to be scored, and the scores, which can be obtained. The criteria used for assigning scores to the tooth surfaces are the same as those use for the OHI (The Oral Hygiene Index).

The OHI-S, like the OHI, has two components, the Debris Index and the Calculus Index. Each of these indexes, in turn, is based on numerical determinations representing the amount of debris or calculus found on the preselected tooth surfaces.

Selection of tooth surfaces

The six surfaces examined for the OHI-S are selected from four posterior and two anterior teeth.

- In the posterior portion of the dentition, the first fully erupted tooth distal to the second bicuspid (15), usually the first molar (16) but sometimes the second (17) or third molar (18), is examined. The buccal surfaces of the selected upper molars and the lingual surfaces of the selected lower molars are inspected.

- In the anterior portion of the mouth, the labial surfaces of the upper right (11) and the lower left central incisors (31) are scored. In the absence of either of this anterior teeth, the central incisor (21 or 41 respectively) on the opposite side of the midline is substituted.

Criteria for classifying debris

Scores	Criteria
0	No debris or stain present
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface.

Criteria for classifying calculus

Scores	Criteria
0	No calculus present
1	Supragingival calculus covering not more than third of the exposed tooth surface.
2	Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
3	Supragingival calculus covering more than two third of the exposed tooth surface or a continuos heavy band of subgingival calculus around the cervical portion of the tooth or both.

Interpretation

Individually DI-S and CI-S is scored as follows:

0.0 to 0.6 = Good oral hygiene

0.7 to 1.8 = Fair oral hygiene

1.9 to 3.0 = Poor oral hygiene

An OHI-S is scored as follows:

0.0-1.2 = Good oral hygiene

1.3 -3.0 = Fair oral hygiene

3.1 -6.0 = Poor oral hygiene

Oral Hygiene Index	=	**Debris Index + Calculus Index**

Gingival Index: [47]

Gingival Index (GI) was introduced by **Loe and Silness** in 1963

- GI could be used in all teeth or selected teeth and in all surfaces or selected surfaces.
- The examination done by blunt probe.
- Partially erupted teeth, retained roots, teeth with periapical lesion and third molars should be excluded and there is no substitution.

Calculation:

GI = Total scores / No. of surfaces examined

If we want to calculate the maximum score for gingival index (4 surfaces and 6 teeth)

Interpretation:

Score Criteria

0 - No inflammation.

1 - Mild inflammation, slight change in color, slight edema, no bleeding on probing.

2 - Moderate inflammation, moderate glazing, redness, bleeding on probing.

3 - Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

GI is scored as follows:

0.1-1: Mild gingivitis

1.1- 2: Moderate gingivitis

2.1- 3: Severe gingivitis

Probing pocket depth (PD): [48]

Probing depth is a measurement of the depth of a sulcus or periodontal pocket. It is determined by measuring the distance from the gingival margin to the base of the sulcus or pocket with a calibrated periodontal probe. Probing involves "stepping" a calibrated periodontal probe (UNC-15) around the tooth and recording the deepest point at each of six tooth surfaces: distofacial, facial, mesiofacial, distolingual,

lingual, and mesiolingual. As a general rule, a probe reading that falls between two calibrated marks on the probe should be rounded upward to the next highest millimetre. Out of 6 surfaces per tooth, the highest probing depth value is taken as the probing depth of that individual tooth.

Clinical Attachment Level (CAL): [48]

CAL is the distance from the cementoenamel junction (CEJ) to the base of the periodontal pocket. CAL measurement also involves measuring with a calibrated periodontal probe (UNC-15) around the tooth and recording the deepest point at each of six tooth surfaces from the CEJ: distofacial, facial, mesiofacial, distolingual, lingual, and mesiolingual.

Collection of saliva:

All thePatients in the study were asked to rinse with normal water (to wash out exfoliated cells) and then wait for 5 minutes. 5 ml of unstimulated saliva was collected from each patients between 9.00am to 11.00am as method given by **Navazesh M 1993.**[49] Unstimulated saliva was collected by the spit method in a sterile sample collection container. The saliva sample was sent to the lab immediately where it was centrifuged at 3000rpm for 5 minutes and then the ALP enzyme activity in saliva was determined spectrophotometrically with the help of a semi-autoanalyser (BTS 350, BIOSYS®) with the IFCC recommendations.

Collection of blood:

5ml of fasting blood samples were collected from each individuals transferred to the nearby biochemical laboratory for assay. After an hour, the supernatant serum was extracted and Total ALP levels were evaluated semi-autoanalyser (BTS 350, BIOSYS®) with the IFCC recommendations and the results expressed in U/L.

Non-surgical periodontal therapy:

Following the sample collections, complete ultrasonic scaling was performed to all the patients in study group. All the patients were instructed to maintain their oral hygiene with Modified Bass brushing technique and to use Chlorhexidine mouth wash twice daily. Root planing, wherever required was done after 15days from baseline within 2 subsequent visits. All the patients were recalled on 30^{th} day following completion of phase 1 periodontal therapy for review and post-operative sample collection (both blood and saliva).

ALP ESTIMATION BY SPECTROMETRY:

Method:

Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Principle:

p-Nitrophenylphosphate + H_20 ⟶ Phosphate + p-Nitrophenol

Reagents:

Components and Concentrations:

R1: 2-Amino-2-methyl-1 -propanol pH 10.4	1.1 mol/L
Magnesium acetate	2 mmol/L
Zinc sulphate	0.5 mmol/L
HEDTA	2.5 mmol/L
R2: p-Nitrophenylphosphate	80 mmol/L

Storage Instructions and Reagent Stability:

The reagents are stable upto the end of the indicated month of expiry, if stored at 2-8 °C and contamination is avoided.

Reagent Preparation:

Substrate Start

The reagents are ready to use.

Sample Start

Mix **4** parts of R1 + 1 part of R2 (e.g. 20 mL R1 + 5 ml_ R2) = monoreagent

Stability:

4 weeks	at	2-8 °C
5 days	at	15-25°C

Specimen

Stability:

7 days	at	20-25°C
7 days	at	4 - 8 °C
2 months	at	-20 °C

Sample start

	Blank	**Sample or calibrator**
Sample or calibrator	-	20 µL
Dlst. Water	20 µL	-
I Monoreagent	1000 µL	1000 µL

Mix, read absorbance after 1 **min.** and start stopwatch. Read absorbance again after 1, 2 and 3 min.

Performance characteristics

Measuring range

On automated systems the test is suitable for the determination of AP activities up to 1400 U/L.

In case of a manual procedure, the test is suitable for AP activitieswhich correspond to a maximum of AA/min of 0.25.

If such values are exceeded the samples should be diluted 1+9with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences:

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated bilirubin up to 60 mg/dL, unconjugated bilirubin to 25 mg/dL, hemoglobin up to 100 mg/dL and lipemia up to 2000 mg/dL triglycerides.

Sensitivity/Limit of Detection:

The lower limit of detection is 2 U/L.

Basic Armamentarium:

1. Mouth mirror
2. UNC 15 probe
3. Universal curettes- 2R/2L & 4R/4L
4. Gracey curettes- #1-14
5. Surgical gloves
6. Mouth masks
7. Tweezers
8. Cotton rolls
9. 5 ml sterile saliva collection containers
10. 2 ml blood collection sample tubes
11. ALP enzyme kit. Diasys® ALP

Figure 1: Chronic generalised periodontitis Pre-operative 1

Figure 2: Chronic generalised periodontitis Pre-operative 2

Figure 3: Chronic generalised periodontitis Pre-operative 3

Figure 4: Probing depth >5mm at baseline

Figure 5: Following non-surgical therapy 1

Figure 6: Following non-surgical therapy 2

Figure 7: Following non-surgical therapy 3

Figure 8: Probing depth after phase 1 periodontal therapy

Figure 9: Collection of blood (I.V)

Figure 10: Collection of saliva

Figure 11: Collected blood sample

Figure 12: Collected saliva sample

Figure 13: Centrifuge machine

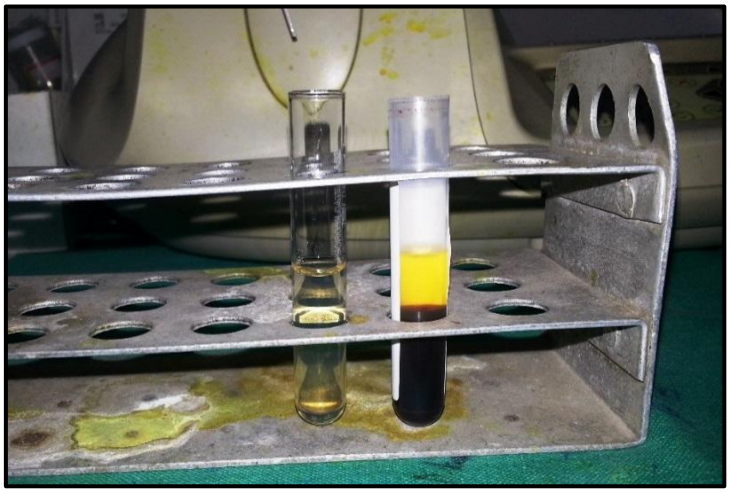


Figure 14: Centrifuged saliva and serum samples

Figure 15: ALP kit (Diasys®)

Figure 16: Semi auto analyser for ALP estimation

Figure 17: Armamentarium

RESULTS

This study was conducted to evaluate the levels of serum and salivary ALP in patients with generalized chronic periodontitis before and after non-surgical periodontal therapy and to compare the outcomes with healthy subjects. A total of 40 chronic generalised periodontitis (study group) and 10 periodontally healthy subjects (control group) were selected from the out-patient section of department of periodontics, Adhiparasakthi dental college and hospital. All clinical parameters were measured at baseline with saliva and blood samples collected on the same day and then 30 days after phase 1 periodontal therapy. Saliva and blood samples were sent for spectrometric analysis for ALP estimation. The obtained results were tabulated and the data collected were subject to statistical analysis.

STATISTICAL ANALYSIS:

The collected data was subjected to statistical analysis through SPSS (Statistical Package for Social Science).

The **Paired t test** was used to assess the baseline and post-operative values of clinical parameters such as OHI-S, Gingival index, probing pocket depth, Clinical Attachment Level and biomarker parameters such as serum ALP and salivary ALP levels.

The paired samples correlations (**Paired t test**) was used to assess the correlation between clinical parameters such as OHI-S,

Gingival index, probing depth, CAL and biomarker parameters such as serum ALP and salivary ALP levels.

The **One way ANOVA** was used to compare the enzyme levels in saliva and serum between the study group at baseline, post-operatively and control group.

Table 1: Mean change in OHI-S score from baseline to post-operative in study group

	Baseline	Post-operative	T value	P-value
OHI-S	3.00 ± .04	.99 ± .02	57.87	.000**

*Significant at the 1% level. **Statistically highly significant difference*

The mean OHI-S score of study group at baseline was **3.00 ± .04** and post-operative score was found to be **99 ± .02**. On comparing baseline score with post-operative score, the reduction in the OHI-S score was found to be statistically significant with P-value .000**

Chart 1: Mean change in OHI-S score from baseline to post-operative in study group

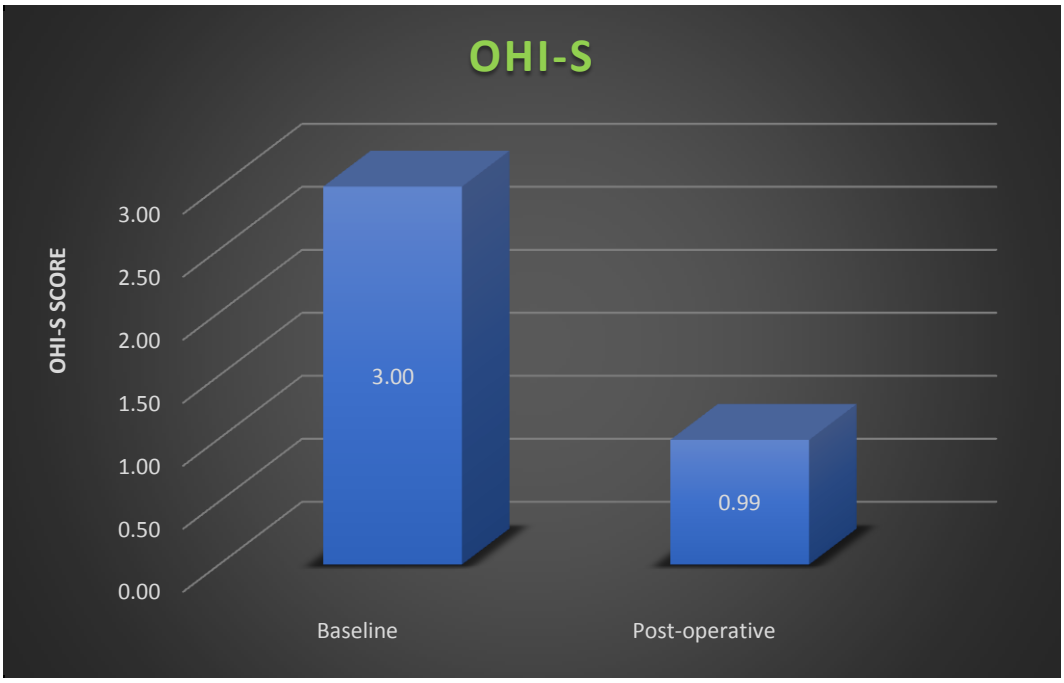


Table 2: Mean change in GI score from baseline to post-operative in study group

	Baseline	Post-operative	T value	P-value
GI	1.97 ± .05	.61 ± .01	26.81	.000**

*Significant at the 1% level. **Statistically highly significant difference*

The mean GI score at baseline in study group was **1.97 ± .05** and post-operative score was found to be **.61 ± .01**. On comparing baseline score with post-operative score, the reduction in the GI score was found to be statistically significant with P-value .000**

Chart 2: Mean change in GI score from baseline to post-operative in study group

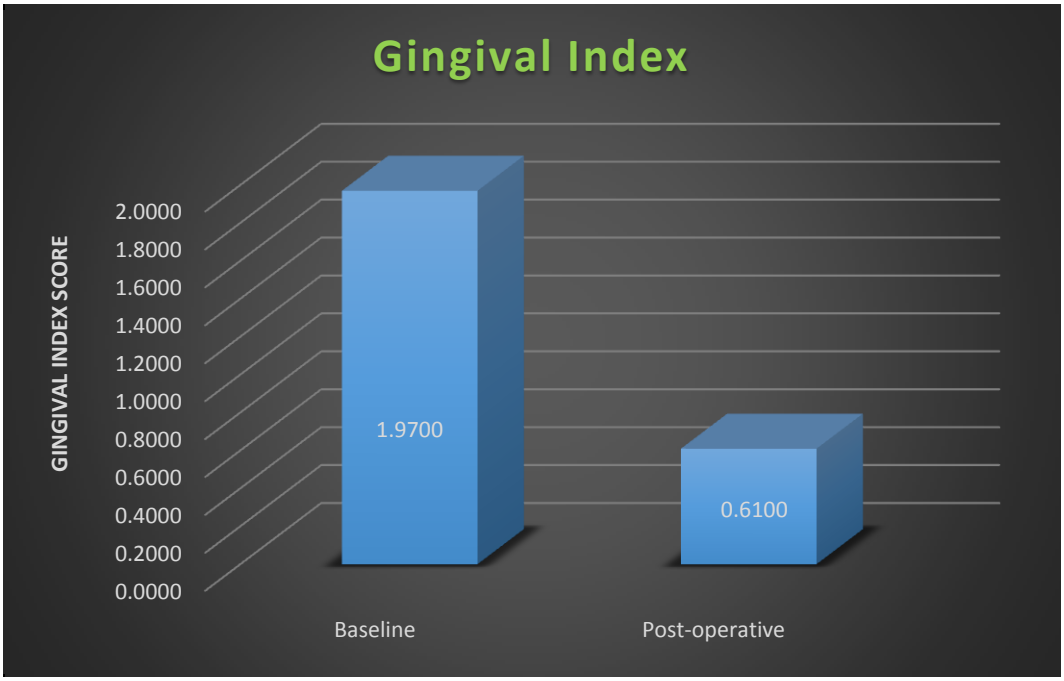


Table 3: Mean change in probing depth & CAL from baseline to post-operative in study group

	Baseline	Post-operative	T value	P-value
PD	3.63 ± .17mm	1.93 ± .09mm	17.88	.000**
CAL	4.14 ± .19mm	2.23 ± .09mm	18.58	.000**

*Significant at the 1% level. **Statistically highly significant difference*

The mean probing depth & CAL in study group at baseline was **3.63 ± .17mm** and **4.14 ± .19mm** respectively and post-operatively **1.93 ± .09mm** and **2.23 ± .09mm**. On comparing baseline values with post-operative values, the reduction in the probing depth and gain in CAL post-operatively was found to be statistically significant with P-value .000**.

Chart 3: Mean change in probing depth & CAL from baseline to post-operative in study group

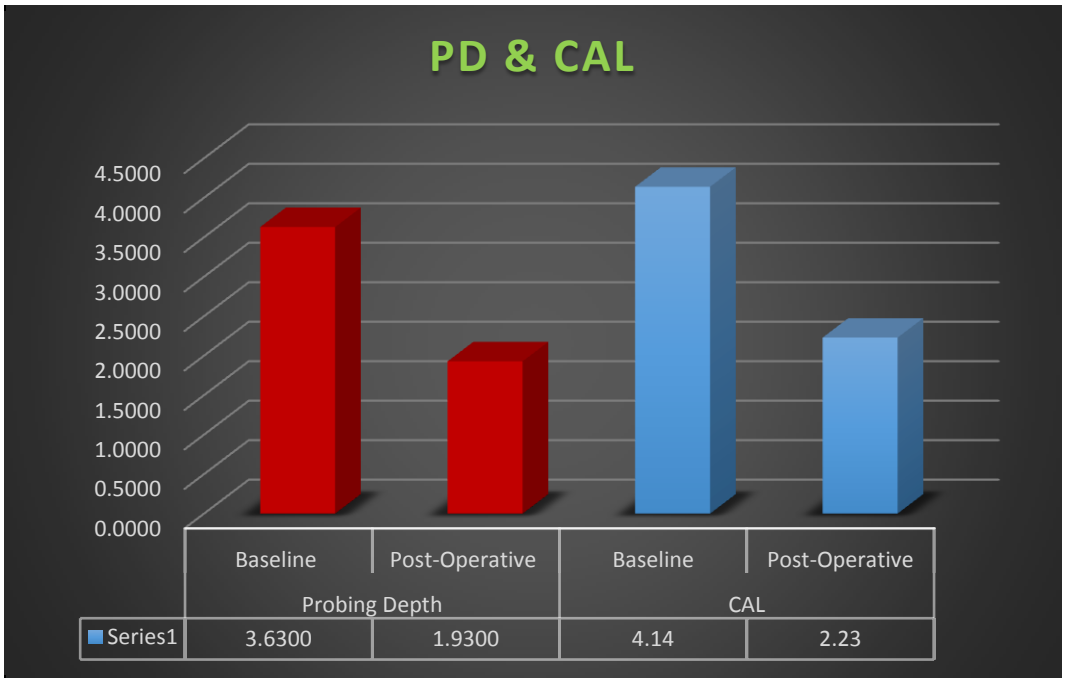


Table 4: Comparison of mean baseline salivary & serum ALP levels between control group and study group

	Control group	Study group	F value	P-value
Saliva ALP	23.00 ± 6.67	79.55 ± 6.40	13.36	.000**
Serum ALP	72.70 ± 2.19	97.62 ± 4.17	4.95	.009**

*Significant at the 1% level. **Statistically highly significant difference*

On Comparing the mean baseline values of salivary andserum ALP levels of control group with baseline values of study group, the difference in salivary and serum ALP levels between control group (23.00 ± 6.67 and 72.70 ± 2.19) and study group (79.55 ± 6.40 and 97.62 ± 4.17) was found to be statistically significant with P-value of .000** and .009** for saliva and serum respectively.

Table 5: Comparison of mean baseline salivary & serum ALP values with post-operative ALP values of study group

	Baseline	**Post-operative**	**F value**	**P-value**
Saliva ALP	79.55 ± 6.40	49.47 ± 5.11	13.36	.000**
Serum ALP	97.62 ± 4.17	85.40 ± 4.10	4.95	.009**

*Significant at the 1% level. **Statistically highly significant difference*

On Comparing the mean baseline salivary and serum ALP values with post-operative values in study group, the difference in salivary and serum ALP levels from baseline (79.55 ± 6.40 and 97.62 ± 4.17) to post-operative (49.47 ± 5.11 and 85.40 ± 4.10) was found to be statistically significant with P-value of .000** and .009** for saliva and serum respectively.

Table 6: Comparison of mean baseline salivary & serum ALP values of control group with post-operative ALP values of study group

	Control group	**Study group Post-operative**	**F value**	**P-value**
Saliva ALP	23.00 ± 6.67	49.47 ± 5.11	13.36	.000**
Serum ALP	72.70 ± 2.19	85.40 ± 4.10	4.95	.009**

*Significant at the 1% level. **Statistically highly significant difference*

On Comparing the mean baseline salivary and serum ALP values of control group with post-operative values of study group, the difference between the ALP levels in baseline of control group (23.00 ± 6.67 and 72.70 ± 2.19) to post-operative of study group (49.47 ± 5.11 and 85.40 ± 4.10) was found to be statistically significant with P-value of .000** and .009** for saliva and serum respectively.

Chart 4: Comparison of mean salivary ALP levels in control group with baseline and post-operative ALP levels of study group

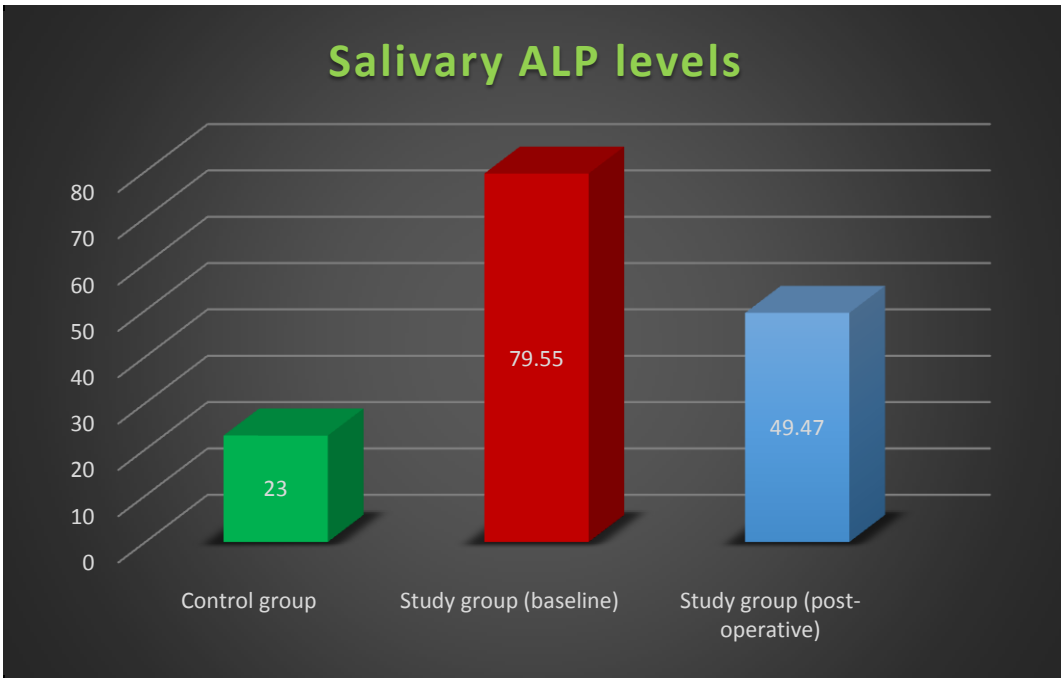
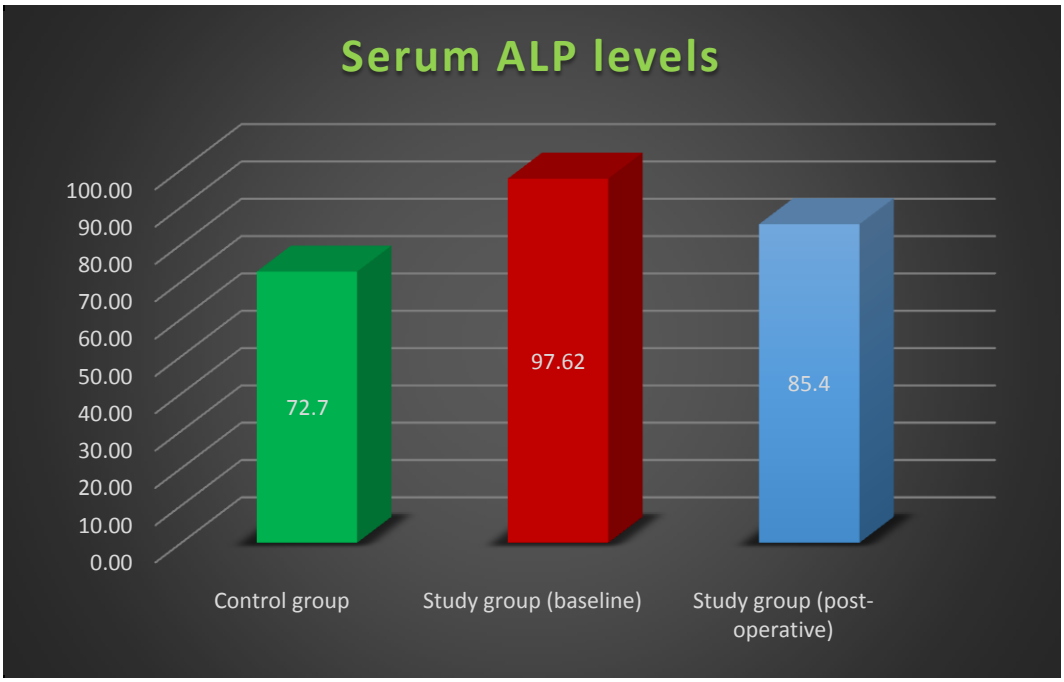


Chart 5: Comparison of mean serum ALP values in control group with baseline and post-operative ALP levels of study group



DISCUSSION

A comparative clinical study was conducted in department of Periodontology, APDCH, Melmaruvathur, Tamilnadu. The study population consists of 50 participants of which, 40 patients with chronic generalised periodontitis (Study group) and 10 periodontally healthy individuals (Control group) were included in the study to whom the study design was explained and informed consent was obtained from all the patients included in the study. Clinical indices including OHI-S, Gingival Index and clinical parameters including Probing depth, CAL were measured at baseline and following phase 1 periodontal for the study group. No clinical parameters were evaluated for the control group, since the control group exhibited <20% of sites bleeding on probingand probing pocket depth of 2-3mm and no attachment loss, along with good oral hygiene maintenance.

Alkaline phosphatase (ALP) levels in serum and saliva were evaluated by collecting the blood and saliva samples from periodontally healthy individuals once and at baseline and then following phase 1 periodontal therapy in patients with chronic generalised periodontitis.

The term ‘biomarker’ refers to biologic substances that can be measured and evaluated to serve as indicators of biological health, pathogenic processes, environmental exposure and pharmacologic responses to a therapeutic intervention. [50]

Therefore, better understanding and thorough knowledge of the biomarkers of health and disease leads to enhanced execution of appropriate and personalized preventive and therapeutic strategies to maintain finest health of an individual.

Periodontal pathogenic processes can be generally divided into three phases: inflammation; connective tissue degradation; and bone turnover. During each phase of the disease, specific host-derived biomarkers have been identified and therefore provide a general sense of stage of pathologic process the patient is currently undergoing.

Among several biomarkers of periodontal disease activity, alkaline phosphatase, being a phenotype marker of bone turnover rate has been found to be elevated in a variety of bone disorders with the highest elevations occur in Paget's disease (osteitisdeformans). Other bone disorders including osteomalacia, rickets, hyperparathyroidism, and osteogenic sarcoma have also shown elevated levels of ALP. In addition, increased levels are also seen in the case of healing bone fractures and during periods of physiologic bone growth.

In the past few years, various cross sectional clinical studies in humans have been conducted and proved the robust relationship between the periodontitis and elevated ALP levels in serum and in GCF.[3, 8, 9] Though predictable, the sampling of blood by intravenous method is invasive and causes discomfort to the patients, its use for periodontal disease is of less patient compliance. And though reliable,

sampling from GCF is technique sensitive and takes longer time comparatively with the sample collection time for saliva.

Saliva, rich in serum albumin and in antimicrobial and immuno-modulary proteins, subsidise the lubrication of oral cavity, buffering of the tooth surface thus maintaining the integrity of the oral cavity. Saliva also contains non-salivary elements, such as gingival crevicular fluid, desquamated cells, nasopharyngeal discharge, extraneous debris, and bacteria and bacterial by-products. [51]

Numerous advantages lies in choosing saliva as a diagnostic tool which includes its readily available nature, it can be collected in a comfortable manner unlike blood collection by venepuncture and the associated fear of the needle, whereas saliva can be collection in a non-invasive manner, which makes patient compliance a reliable factor.

Various studies in the past few years have revealed the potential to identify and measure numerous biomarkers in saliva for the diagnosis of periodontal diseases and monitoring its progression and health.

The study conducted by **Miglani D C et al., in 1974** [28] revealed the relationship between periodontal disease and ALP levels in saliva was the first study in Indian population, correlating the Periodontal disease status with salivary ALP levels. Later, studies by **Todorovic T et al., in 2006** [7] showed that the activity of ALP along with various other enzymes in saliva was increased in patients with periodontal

disease with a positive correlation with gingival index, when compared with periodontally healthy individuals. Further study by **Desai S et al., in 2008** [31] evaluated ALP levels in unstimulated saliva of 120 patients with chronic periodontitis which were correlated with CPITN index. The study results showed that the ALP levels in saliva was increased with the increase in the CPITN score.

Dabra S et al., in 2012 [37] evaluated the **s**alivary levels of ALP activity in patients with periodontal disease, before and after periodontal treatment resulted that there was significantly increased activity of ALP in saliva from patients with periodontal disease and also a significant reduction in the enzyme levels after conventional periodontal therapy.

Trivedi D et al., in 2012 [39] estimated the ALP level along with other enzymes in unstimulated saliva for the diagnosis of periodontitis in patients with chronic periodontitis and healthy individuals. The results of the study signified increased activities of ALP and other enzymes in unstimulated saliva of patients with chronic periodontitis.Study by **Ramesh A et al., in 2013** [5] estimated the salivary ALP levels in post-menopausal women with and without chronic periodontitis and the results showed a significant increase in salivary ALP levels in post-menopausal women with chronic periodontitis. Later, **Luke R et al., in 2015** [44] estimated the levels of ALP along with other few enzymes in saliva and correlated it with that of clinical parameters in healthy subjects, gingivitis patients and

patients with chronic periodontitis and the results showed significantly increased activity of ALP along with the other enzymes in saliva from patients with periodontal disease than that of gingivitis and control group.

All the studies conducted so far has aimed to rationalise the use of ALP as a biomarker in diagnosing the disease activity of periodontitis. The studies also have established the use of either saliva or serum or even GCF solely in evaluating the ALP levels in patients with chronic periodontitis and even comparison of the same with periodontally healthy individuals. But none of the studies have compared the serum and salivary ALP levels in chronic periodontitis patients and periodontally healthy individuals of same age group to validate the fact that saliva could be used over serum and GCF to assess the disease activity in patients with chronic periodontitis and also its significance of increased activity with periodontally healthy individuals.

Hence, the present study was conducted to compare and evaluate the ALP levels in serum and saliva in patients with chronic periodontitis and to compare the ALP levels with that of the healthy individuals. All clinical parameters including probing depth, CAL and indices including OHI-S and GI, along with serum and salivary samples were collected at baseline, and then 30 days after completion of phase 1 periodontal therapy.

The results indicated that there was a significant reduction in the mean OHI-S scores in the chronic periodontitis group from baseline to 30 days from **3.00 ± .04 to .99 ± .02** post-operatively. This implies the good oral hygiene maintenance of the patient following periodontal phase 1 therapy, which is of utmost importance factor in the prognosis of the treatment outcomes.

There is a significant reduction in the mean Gingival Index scores from baseline to 30 days from **1.97 ± .05 to .61 ± .01** following periodontal phase 1 therapy which is in accordance with the study conducted by **Todorovic T et al., in 2006**. [7] This may be due to the elimination of local etiological factors which harbours numerous pathogenic strains

There is a significant reduction in mean probing pocket depth readings from baseline to 30 days from **3.63 ± .17mm** to **1.93 ± .09mm** following periodontal phase 1 therapy. This can be attributed to the scaling and root planing, as it leads to resolution of inflammatory response and cessation of periodontal disease progression, thereby resulting in reduction of probing depth which is in accordance with the studies conducted by **Daltaban Ö et al., 2006** [30] **and Todorovic et al., 2006.** [7]

There is a significant reduction in mean CAL readings from baseline to 30 days from **4.14 ± .19mm** to **2.23 ± .09mm** following periodontal phase 1 therapy which is in accordance with the study done by **Dabra S et al., 2012.** [37] The CAL gain may be due to effectiveness

of Scaling and root planing as it leads to resolution of inflammatory response and cessation of periodontal disease progression and hence resulting in a relative gain of clinical attachment level. Scaling and root planing also helps in eliminating the bacteria present at the site, thus reducing the colonization of periodontal pathogens resulting in making a favourable environment for oral hygiene maintenance by the patient.

Following phase 1 periodontal therapy, the patients with chronic periodontitis has shown a proportionate reduction in the ALP mean values in both saliva (**79.55 ± 6.40** to **49.47 ± 5.11**) and serum (**97.62 ± 4.17** to **85.40 ± 4.10**) respectively. The reduction in the values may be due to thorough scaling and root planing with proper oral hygiene maintenance of the patients. The results of this study is in accordance with the results obtained from the study conducted by **Dabra S et al., in 2011** [37] for salivary ALP levels at baseline and post-operatively, which was **(29.43±13.10** to **44.55±19.14**).

In the present study, treatment instituted for the patients with chronic periodontitis which included scaling and root planing. Following phase 1 periodontal therapy, all the patients received oral hygiene instructions for home care plaque control, which lead to improvement in clinical parameters. Along with the clinical parameters, the salivary and serum ALP levels were shown to be decreased significantly following phase 1 periodontal therapy due to the reason that the collagen breakdown and alveolar bone resorption

caused by the active inflammation gets subsided with reduction in inflammation following phase 1 periodontal treatment. In addition to the salivary and serum ALP levels obtained from chronic periodontitis group, both serum and salivary ALP levels were also evaluated in 10 periodontally healthy volunteers with no clinical signs of gingival or periodontal disease, which were **23.00 ± 6.67** for saliva and **72.70 ± 2.19** for serum which was found to be statistically significant when compared with the mean values of the baseline of chronic periodontitis group with the P-value of .009** and .000** for saliva and serum respectively.

Utilization of saliva as diagnostic fluid has got various advantages like painless technique, non-invasive, ease of collection and storage, cost-effective and readily available for any diagnostic purposes. Hence, in the present study, saliva have been chosen to evaluate the ALP levels and to compare the values with that of serum to validate that saliva could be chosen over serum for biomarker evaluation.

In addition, we chose ALP as a periodontal disease marker because of its role potential behaviour in the periodontal disease progression of an individual. ALP levels were found to be higher during collagen breakdown and alveolar bone resorption caused by periodontal disease. Various clinical studies have proved the beneficial outcome of choosing ALP as periodontal disease marker and its good predictable value. Also, the evaluation of ALP in laboratory is cheaper

and the method of evaluation by spectrophotometry is made simplified by the advances in equipment to auto/ semi-auto analyser. Hence, ALP can be used to rule out the periodontal disease activity in a short period of time. Another advantage of electing ALP as a biomarker of periodontal disease progression is because of its low cost, which will be much useful for patients with poor socio-economic status.

The results of the present study signifies that there is substantial improvement in the mean values of clinical indices which includes OHI-S, Gingival Index, clinical parameters including Probing pocket depth, CAL following phase 1 periodontal therapy that includes complete scaling and thorough root planing. Interestingly, the mean ALP values in both saliva and serum also exhibited significant reduction following the phase 1 periodontal therapy, which shows a positive correlation with the mean values of clinical parameters and indices.

On comparing the mean baseline salivary and serum ALP values with post-operative ALP values in study group, the difference in salivary and serum ALP levels from baseline to post-operative was found to be statistically significant. The mean ALP values in saliva and serum of control group (periodontally healthy individuals) when compared to the study group (chronic generalised periodontitis) the difference in ALP levels in both baseline and post-operatively was also found to be statistically significant.

This is the first study to compare and evaluate the ALP levels in saliva and serum in patients with chronic generalised periodontitis and to compare the ALP levels with that of the healthy individuals. In addition, the clinical indices including OHI-S, GI, and clinical parameters including probing depth and CAL was also compared for the patients with chronic generalised periodontitis at baseline and after phase 1 periodontal therapy to evaluate the treatment outcomes following phase 1 periodontal therapy in chronic periodontitis patients.

With the results of the present study, it could be suggested that salivary ALP levels could be used instead of serum ALP levels to evaluate the periodontal disease activity in patients with chronic generalised periodontitis. Further studies with large population size should be conducted to synergize the outcome of the present study.

CONCLUSION

The traditional method of diagnosing periodontitis one includes assessment of clinical parameters and radiographic aids to evaluate the periodontal tissue destruction.

However screening and diagnostic modalities for the early identification of periodontitis is not significant through the conventional diagnostic method, a need for evolution of reliable diagnostic methods to rule out periodontal disease in the early stage of the disease progression lead to the emergence of biomarkers in the field of diagnosis.

So far, several inflammatory and immune mediators have been implicated as biomarkers in the periodontal tissue destruction in both saliva and serum and shown its clinical predictability of recognizing disease activity

Alkaline phosphatase (ALP), being a hydrolase enzyme which is also considered as a phenotypic marker is a major destructive enzyme involved and expressed in high concentrations during the degradation and remodelling of alveolar bone and collagenase activity of the periodontium

Evaluation of ALP by simplified method of spectrometry and its cheaper analysis cost makes the biomarker ALP as a resilient and reliable one for the diagnosis of periodontal disease activity for any individual

Serum has the potential to be used as the diagnostic fluid for oral diseases and is usually used for the detection of active face of periodontal disease to identify individuals at higher risk for future disease occurrence. With the advent of highly sensitive techniques, traces of markers can be accurately established in serum

Saliva has the potential to be used as the diagnostic fluid for oral disease. Its easy method of collection through non-invasive methods, without specialized equipment or personnel, and also contains locally derived and systemically derived markers and also its readily availability makes saliva to choose over serum for the evaluation of biomarkers

The results of present study shown that

1. ALP levels in saliva and serum were increased in patients with chronic generalized periodontitis when compared with periodontally healthy individuals.
2. The increase in the salivary and serum ALP levels were positively correlated with the clinical indices and parameters including OHI-S, Gingival index, probing depth and CAL in study group.
3. Following the phase I periodontal treatment there was a significant decrease in the salivary and serum ALP levels in patients with chronic generalized periodontitis along with improvement in the clinical parameters.

4. The ALP activity during disease progression and following phase I periodontal treatment in saliva and serum were also positively correlated with respect to disease course.

With the limitations of the present study it could be concluded that ALP levels in saliva could be evaluated for the diagnosis of active phase of periodontal disease and also for evaluation of the treatment outcomes following periodontal phase I therapy. Further study with large sample size and with different duration of ALP estimation in saliva should be done to support the evidence of the present study.

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PROFORMA

EVALUATION OF SERUM AND SALIVARY ALKALINE PHOSPHATASE LEVELS IN PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY: A COMPARATIVE STUDY

S.no: OP.No: Date:

Name: Age: Gender: Address:

Occupation:

Chief complaint:

History of presenting illness:

Past medical history:

Overall systemic health status report from General medicine OPD:

Past dental history:

Personal history:

1. Oral hygiene measures:

2. Habits:

Intra-Oral examination:

Missing teeth:

Dental caries:

Clinical Parameters (Baseline)

Indices:

OHI-S Index: (Greene & Vermillion-1964)

Debris						
Tooth	16	11	26	36	31	46
Calculus						

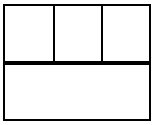
Debris Index = / 6 =

Calculus Index = / 6 =

Oral Hygiene Index	=	**Debris Index + Calculus Index**

Good / Fair / Poor

Gingival index: (Loe and Silness-1963)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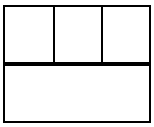
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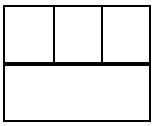
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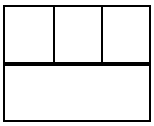
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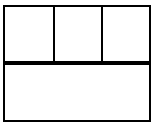
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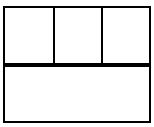

36 =

Gingival Index(GI) = =

24

Mild gingivitis/Moderate gingivitis/ Severe gingivitis

Probing depth:

palatal palatal palatal

17	16	15	14	13	12	11	21	22	23	24	25	26	27

Buccal labial buccal

Lingual lingual lingual

47	46	45	44	43	42	41	31	32	33	34	35	36	37

Buccal labial buccal

CAL:

palatal palatal palatal

17	16	15	14	13	12	11	21	22	23	24	25	26	27

Buccal labial buccal

Lingual lingual lingual

47	46	45	44	43	42	41	31	32	33	34	35	36	37

Buccal labial buccal

Biomarker evaluation:

Serum ALP:

Salivary ALP:

Investigations:

Radiograph:

Others:

Diagnosis:

Treatment:

Date	Treatment done	Appointment date	Staff signature

Clinical Parameters (post-operative)

Indices:

OHI-S Index: (Greene & Vermillion-1964)

Debris						
Tooth	16	11	26	36	31	46
Calculus						

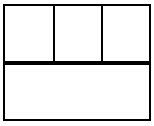
Debris Index = / 6 =

Calculus Index = / 6 =

Oral Hygiene Index	=	**Debris Index + Calculus Index**

Good / Fair / Poor

Gingival index: (Loe and Silness-1963)



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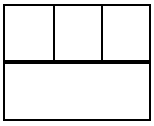

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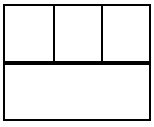

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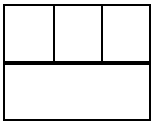
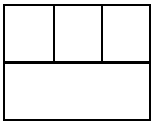
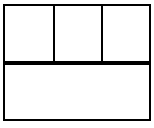


44 =



32 =



36 =

Gingival Index(GI) = =

24

Mild gingivitis/Moderate gingivitis/ Severe gingivitis

Probing depth:

palatal palatal palatal

17	16	15	14	13	12	11	21	22	23	24	25	26	27

Buccal labial buccal

Lingual lingual lingual

47	46	45	44	43	42	41	31	32	33	34	35	36	37

Buccal labial buccal

CAL:

palatal palatal palatal

17	16	15	14	13	12	11	21	22	23	24	25	26	27

Buccal labial buccal

Lingual lingual lingual

47	46	45	44	43	42	41	31	32	33	34	35	36	37

Buccal labial buccal

Biomarker evaluation:

Serum ALP:

Salivary ALP:

PARTICIPANT INFORMED CONSENT FORM (PICF)

(English)

Protocol / Study number: _____

Participant identification number for this trial: _____

The contents of the information sheet dated that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from APDCH. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

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(Signatures / Left Thumb Impression) Signatures of the Principal Investigator

<u>ஆராய்ச்சியில் பங்கேற்பதற்கு இணக்கம்</u>

தேதி:

நோயாளியின் பெயர் :

வயது / பாலினம் :

புறநோயாளி எண் :

அறுவை சிகிச்சை மருத்துவ நிபுணரின் பெயர் :

சிகிச்சையின் பெயர் :_____

அளிக்கப்படும் மயக்க மருந்தின் வகை:

எனது தற்போதைய வாய்நலம் குறித்தும், அதற்கு உரிய சிகிச்சை முறைகளையும், மாற்று சிகிச்சை முறைகளையும் மற்றும் சிகிச்சை மேற்கொள்ளாவிடில் ஏற்படும் பின்விளைவுகளையும் பல் மருத்துவர் முழுமையாக என்னிடம் கூறினார். அதற்கான எனது சந்தேகங்களையும் பல்மருத்துவரிடம் கேட்டு தெளிவுபடுத்தி கொண்டேன். மேலும் சிகிச்சை முறை, என் சிகிச்சையின் போது தேவைப்படும் மயக்கமருந்துகள் மற்றும் பிறமருந்துகள் செலுத்த சம்மதிக்கின்றேன். நான் மனப்பூர்வமாக எனது சிகிச்சை முறை மற்றும் அதனால் வரும் பின்விளைவுகளையும் ஏற்றுக்கொள்கிறேன் மற்றும் மருத்துவர் கூறும் அறிவுரைகளையும் கடைபிடிப்பேன்.

மேலே சொல்லப்பட்டு இருக்கும் ஆராய்ச்சி ஆய்வில் பங்கேற்பதற்கு மனப்பூர்வமான எனது சம்மதம்.

மேலுள்ள தகவல்கள் உள்ளிட்டு ஆராய்ச்சி ஆய்வானது வாய்வழியாக விளக்கப்பட்டிருக்கிறது மற்றும் பங்கேற்பதற்கு சுயவிருப்பத்தில் இணங்குகிறேன் என்பது இந்த ஆவணத்தில் கையெழுத்திடுவதன் அர்த்தமாகும்.

நோயாளியின் கையொப்பம் அறுவை சிகிச்சை நிபுணரின் கையொப்பம்

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ADHIPARASAKTHI DENTAL COLLEGE AND HOSPITAL

Melmaruvathur, Tamilnadu-603019

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MEMBER SECRETARY

Dr.S.Meenakshi, PhD

This ethical committee has undergone the research protocol submitted by Dr.M.J.Renganath, Post Graduate Student, Department of Periodontology under the title "**Evaluation of serum and salivary alkaline phosphatase levels in patients with chronic periodontitis before and after non-surgical periodontal therapy: a comparative study**" Reference No: **2014-MD-BrII-SAS-05,** under the guidance of Prof.Dr.T.Ramakrishnan for consideration of approval to proceed with the study.

This committee has discussed about the material being involved with the study, the qualification of the investigator, the present norms and recommendation from the Clinical Research scientific body and comes to a conclusion that this research protocol fulfils the specific requirements and the committee authorizes the proposal.

Member secretary

Date: